Amendments to the Specifications:

Please replace the paragraph starting at page 4, line 11 of the specification with the following paragraph:

According to the present inventions a method for detecting and/or monitoring cartilage degradation is provided. The method enables such detection by measuring in a biological sample a collagen type II fragment wherein all or a relevant part of the amino acid sequence ~~HRGYPGLDG~~(SEQ ID NO: 1) is contained.

Please replace the paragraph starting at page 4, line 23 of the specification with the following paragraph:

Thus the present invention provides a method of qualitative or quantitative assay or collagen type II or fragments thereof in a biological sample comprising contacting said fragments with an immunological binding partner which is immunoreactive with an epitope comprised in the amino acid sequence ~~HRGYPGLDG~~(SEQ ID NO: 1) and detecting resulting immunoreaction.

Please replace the paragraph starting at page 4, line 30 of the specification with the following paragraph:

The detection performed in the method of the present invention may be carried out with an immunoassay utilizing an antibody, which recognizes an epitope within the collagen type II derived sequence ~~HRGYPGLDG~~(SEQ ID NO: 1) or consisting of the whole of said sequence. To ensure monitoring of collagen type II degradation, a preferred embodiment provides an antibody, which only recognizes the unwound form of the epitope, and not the wound form.

Please replace the paragraph starting at page 5, line 7 of the specification with the following paragraph:

The invention also includes a cell line for production of monoclonal antibodies recognizing an epitope comprised in the collagen type II derived sequence ~~HRGYPGLDG~~(SEQ ID NO: 1).

Please replace the paragraph starting at page 5, line 10 of the specification with the following paragraph:

To employ the present invention a kit utilizing an antibody, which recognizes an epitope comprised in the collagen type II derived sequence ~~HRGYPGLDG~~(SEQ ID NO: 1), together with a suitable detection system, is provided. Supplements to such a kit are a second antibody and a synthetic peptide resembling the epitope. For detection such supplements can be labelled. The kit of the present invention can be applied to samples like mammalian body fluids, extracts from cells or tissues or supernatants from cells or tissues cultured in vitro.

Please replace the paragraph starting at page 6, line 29 of the specification with the following paragraph:

In one embodiment of the present invention, collagen type II fragments containing all or a relevant part the following sequence ~~HRGYPGLDG~~(SEQ ID NO: 1) are detected in a biological sample to enable detection and monitoring of cartilage degradation. Detection of such collagen type II fragments can for example be performed using HPLC, mass spectroscopy, sequencing, or immunoassays. The ~~HRGYPGLDG~~(SEQ ID NO: 1) sequence is unique for the collagen type II chain and is located in the helical part of collagen type II (position 289-297 GeneBank accession nr. NP_001835 isoform 1 and position 220-228 GeneBank accession nr. NP_149162 isoform 2).

Please replace the paragraph starting at page 7, line 9 of the specification with the following paragraph:

Fragments of collagen type II containing the epitope of the ~~HRGYPGLDG~~(SEQ ID NO: 1) sequence vary in size below 80 kDa. Smaller fragments, which can be excreted into urine, are detected in one embodiment of the present invention. These fragments may be smaller than 30 kDa or even more preferred smaller than 10 kDa.

Please replace the paragraph starting at page 7, line 15 of the specification with the following paragraph:

One preferred method of detection is the use of an immunoassay, utilizing an antibody, which binds to an epitope on type II collagen or fragments thereof containing an epitope within the following sequence ~~HRGYPGLDG~~(SEQ ID NO: 1). Assay forms in which such an antibody can be applied include, but are not limited to, ELISA, microarray, RIA, FACS, Western blotting, chromatography, and histochemistry.

Please replace the paragraph starting at page 8, line 1 of the specification with the following paragraph:

In a further embodiment, the invention provides a method for detecting the amount of ~~HRGYPGLDG~~(SEQ ID NO: 1) epitope containing collagen type II derived fragments in urine or serum. A urine sample is contacted with an antibody specific towards an epitope within the amino acid sequence ~~HRGYPGLDG~~(SEQ ID NO: 1), essentially all collagen type II fragments in urine containing this epitope will be bound by such an antibody. The amount of fragments bound by the antibody will be detected by methods well known in the art.

Please replace the paragraph starting at page 8, line 10 of the specification with the following paragraph:

Typically, the epitope bound by antibodies reactive with ~~HRGYPGLDG~~(SEQ ID NO: 1) may comprise five or more amino acids, e.g. the first five amino acids of the sequence.

Please replace the paragraph starting at page 8, line 13 of the specification with the following paragraph:

In a preferred embodiment for measuring cartilage degradation the antibody utilized for detection only recognizes the unwound form of collagen type II or fragments thereof and not the wound form. It will be possible, in tissue or synovial fluid samples for example, to access a ratio between unwound and wound collagen type II or fragments thereof, this can be related to the collagenase activity in the joint from which the sample has been retrieved. Denatured helical collagen domains might be retained in the tissue by cross-linking and fibrillar packaging. This may complicate detection according to the present invention in cartilage tissue samples. To address this problem, the biological sample may first be contacted with an enzyme having the ability to selectively cleave unwound collagens without cleaving the ~~HRGYPGLDG~~(SEQ ID NO: 1) epitope. Such enzymes could be, but is not limited to, trypsin or chymotrypsin, which are unable to cleave wound collagen. The fragments of unwound collagen are then extracted from the biological sample to produce an extract of unwound collagen fragments. This extract can then be assayed as mentioned in the above.

Please replace the paragraph starting at page 10, line 5 of the specification with the following paragraph:

Antisera will be screened for their ability to bind an epitope within the ~~HRGYPGLDG~~(SEQ ID NO: 1) sequence. Their specificity between unwound and wound collagen type II or fragments

thereof, as well cross reactivity with other collagens will be assessed. Antisera from the most promising hosts may be used in their crude form or purified.

Please replace the paragraph starting at page 10, line 11 of the specification with the following paragraph:

Monoclonal antibodies may be generated from immunised mice with the most promising antibody titre, by fusing lymphocytes isolated from the spleen of these mice with a myeloma cell line. The generated hybridoma clones are screened for antibodies with reactivity toward an epitope within the ~~HRGYPGLDG~~(SEQ ID NO: 1) sequence, and cell lines can be established for production and purification of monoclonal antibodies.

Please replace the paragraph starting at page 10, line 22 of the specification with the following paragraph:

One embodiment of the present invention constitutes the development of a diagnostic kit for use in detection and/or monitoring of cartilage degradation. This includes an antibody recognizing an epitope comprised in the following sequence ~~HRGYPGLDG~~(SEQ ID NO: 1), located in type II collagen or fragments thereof, preferably the antibody recognizes unwound collagen type II and not the wound form. Most preferred are antibodies of the present invention, either alone or with a second antibody with specificity towards the first antibody or another part of the epitope containing fragment. The kit can be applied on mammalian body fluids or extracts of cells or tissues, preferably derived from humans. For competition detections a peptide between 6 and 20 amino acids, in which a succession of amino acids is equivalent to the binding epitope for one of said antibodies, might be supplied either in a labelled or non labelled form. The antibodies may be labelled by joining them, either covalently or non-covalently, with a reporter molecule. Suitable reporter molecules or labels, which may be used for ease of detection, include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, magnetic

particles, and the like. One of the non-labelled antibodies or a peptide of the kit might be immobilised, preferably on a solid surface like a micro-titter plate, possibly by conjugation to a suitable protein carrier like BSA.

Please replace the paragraph starting at page 11, line 25 of the specification with the following paragraph:

FIG. 2 shows competitive inhibition of antiserum coll2-1 D3 binding to ~~HRGYPGLDG~~(SEQ ID NO: 1) coated plates using ~~HRGYPGLDG~~(SEQ ID NO: 1) (●), native type II collagen (■), type I collagen ( ) and BSA (♦) as competitors. B/Bo represents the ratio between antibody bound to coated antigen in the presence of competitor antigen (B) or in the absence of competitor antigen (Bo) and is given in percentage;

Please replace the paragraph starting at page 12, line 19 of the specification with the following paragraph:

A sequence of nine amino acids (His-Arg-Gly-Tyr-Pro-Gly-Leu-Asp-Gly-) (SEQ ID NO: 1) derived from the triple helical region of type II collagen [(α1)II] was synthesized using standard Fmoc solid-phase peptide synthesis (HBTU/HOBt protocol) (Chan & White, 2000).

Please replace the paragraph starting at page 13, line 5 of the specification with the following paragraph:

Five antisera, identified as Coll2-1 D1, D2, D3, D4 and D5, was obtained and their specificity were tested with the competitive inhibitiors ~~HRGYPGLDG~~(SEQ ID NO: 1), native type II collagen, type I collagen and BSA.

Please replace the paragraph starting at page 13, line 11 of the specification with the following paragraph:

A competitive immunoassay was developed to quantify breakdown products of type II collagen containing following sequence ~~HRGYPGLDG~~(SEQ ID NO: 1). Synthetic ~~HRGYPGLDG~~(SEQ ID NO: 1) peptides were conjugated to BSA by BS^3 [Bis(sulfosuccininimidyl) suberate, Pierce, Rockford, USA]. The conjugated peptides were coated to microtiter plates (NUNC, Denmark) at 50 ng/ml in 0.08 M $NaHCO_3$ pH 9.6 for at least 48 hours at 4°C. The coated microtiter plates were saturated with 400 µl/wells of saturation buffer (KH_2PO_4 1.5 mM, Na_2HPO_4 8 mM, KCl 2 mM, NaCl 138 mM, BSA 0.5%, lactose monohydrate 5.3% ph 7.2) for 90 minutes at room temperature. Fifty µl of either calibrators (to generate a standard curve), controls or unknown samples, diluted in Ultroser G (Gibco) were pipetted into appropriate wells in the microtiter plate, followed by 100 µl antiserum (see above) diluted 1/40000. Samples were mixed by rotating the plate and incubated 1 hour at room temperature. After three successive washings with washing buffer (Tris 25 mM, NaCl 50 mM pH 7.3), 100µl of horseradish peroxidase-conjugated goat antibodies to rabbit IgG (Biosource, Belgium) were added to each well and incubated 1 hour at room temperature. After another washing step, 100µl of freshly prepared enzyme substrate (TMB, Biosource, Belgium) were added to each well. After 15 minutes incubation, the reaction was stopped with 100 µl 4M H_3PO_4. The absorbance was read with a microplate reader (Labsystem iEMS Reader MF, Finland) at 450 nm and corrected for absorbance at 620 nm. A standard curve was constructed on a log-linear graph by plotting the B/Bo of 6 calibrators (2000 to 10 nM) (FIG. 1). The concentration of ~~HRGYPGLDG~~(SEQ ID NO: 1) containing peptides in the unknown samples and controls, were determined by interpolation on the calibration curve.

Please replace the paragraph starting at page 14, line 14 of the specification with the following paragraph:

The antisera produced, were tested for their specificity for ~~HRGYPGLDG~~(SEQ ID NO: 1), by use of the immunoassay described in example 1. To test for specificity ~~HRGYPGLDG~~(SEQ ID NO: 1) peptide, collagen type II, collagen type I or BSA, was added in increasing concentrations.

Please replace the paragraph starting at page 14, line 19 of the specification with the following paragraph:

Native type II collagen, type I collagen and BSA, was not able to compete with the coated ~~HRGYPGLDG~~(SEQ ID NO: 1) peptide in the applied concentrations, shown for Coll2-1 D3 in FIG. 2.

Please replace the paragraph starting at page 15, line 2 of the specification with the following paragraph:

Serum from three patients with OA, which were candidates for hip or knee prosthesis, was assayed for ~~HRGYPGLDG~~(SEQ ID NO: 1) containing collagen type II or fragments thereof. The assays were repeated 10 times to assess the intra-assay coefficient of variation. The CV calculations were performed as follows (SD/Mean concentration)*100%.

Please replace the paragraph starting at page 16, line 2 of the specification with the following paragraph:

A serum sample was spiked with known concentrations of synthetic ~~HRGYPGLDG~~(SEQ ID NO: 1) peptide, to ensure that its presence would not effect the recovery of collagen type II or fragments thereof present in the serum sample.

Please replace the paragraph starting at page 16, line 11 of the specification with the following paragraph:

As already shown in the specificity assay of example 2, Coll2-1 D3 does not bind native (wound) collagen type II, as this is not able to compete with the antiserum binding to coated ~~HRGYPGLDG~~(SEQ ID NO: 1) peptide. In the following example digestion of cartilage with collagenase A from *Clostridium histolyticum*, was used to asses the ability of Coll2-1 D3 to bind unwound collagen type II compared to wound collagen type II (FIG. 3).

Please replace the paragraph starting at page 17, line 13 of the specification with the following paragraph:

Sera from healthy volunteers and patients were collected and subjected to the assay described in example 1, utilizing antiserum Coll2-1 D3. The concentration in nM of ~~HRGYPGLDG~~(SEQ ID NO: 1) containing collagen type II or fragments thereof looked as follows: